NOTES ICHTYOLOGIQUES

USE OF TRYPSIN TO EXTRACT AND EXAMINE LARVAL FISH OTOLITHS.

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RÉSUMÉ. - L'extraction et l'observation des otolithes des larves de corégonidés sont considérablement facilitées par une digestion préalable du neurocrane dans une solution de trypsine pendant 1 heure à 28°C.

Key-words. - Coregonidae, *Coregonus lavaretus*, Otolith removal, Trypsin, Larvae.

Since Pannella (1971) first established that daily growth rings occur in the otoliths of many teleost fish, several investigators have evaluated the relationship between these growth increments in fish larvae and their age and a range of physical (temperature) and biological variables (hatching, yolk-sac absorption, exogenous feeding, starvation). For recording the early life history and/or monitoring the mass-marking of fish larvae, otoliths can be observed without special preparation (Campana and Neilson, 1985; Brothers, 1987).

The three pairs of otoliths of the embryos and larvae of *Coregonus lavaretus* L. are directly visible, using a binocular dissecting microscope (transmitted-light at 10X-40X), due to the transparency of the otic capsules at the base of brain. In eleuthero embryos (total length 10 mm), the sagittal otoliths of this species are larger and about 60 µm in diameter. To investigate the internal structure of otoliths in larval fish it is necessary to remove and prepare these small structures by dissection. Otolith removal from larval fishes obviously requires special care and dissection must be performed with very fine, sharp needles. For an experienced person, the standard dissection and mounting process takes 10 to 15 minutes per larva. In this paper, we propose a method to reduce the process to less than 5 minutes.

The whole neurocranium (without eyes and branchials) is immersed in a trypsin solution (100-200 µl/ml) derived from porcine pancreas (EC 3.4.21.4.) for 1 hour at 28°C. After this time, all the digested material floats on the surface and the otoliths sink to the bottom from which they are relatively easy to remove with a syringe.

In contrast with classical dissection that often requires the use of chemicals such as sodium hypochlorite, hydrogen peroxide or ultrasonic cleaning (Brothers, 1987), the otoliths obtained by the trypsin method do not need any further preparation (Fig. 1). They are very clean and the analysis of the microstructure is greatly facilitated. Whereas some other methods use decalcification of the otolith (e.g. Zhang *et al.*, 1991) to improve the examination of the daily growth increments, trypsin probably has a hydrolytic action on the otolin protein as defined by Degens *et al.*, (1969). This partial action improves significantly (Fig. 1) the examination of the daily growth increments and also enhances the visualisation of any labelling, for example, by tetracycline treatments as used by Nagiec *et al.*, (1988). Moreover, this new technique has no effect on ring counts. If otoliths are immersed for an extended period (≥ 12 hours), they disappear due to the digestion of the entire protein matrix. The immersion time is a function of the type and size of the otolith and must therefore be determined for each species of larval fish. The technique can be applied to any shape of otolith. The new technique thus appears to have significant advantages in comparison with earlier methods and, in addition, does not result in the slight shrinkage described by Zhang *et al.* (1991).

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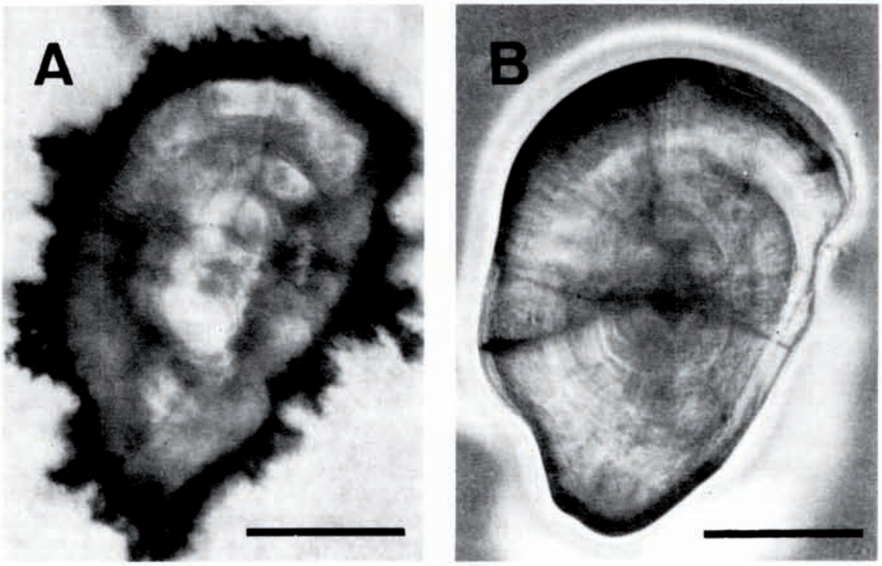

Fig. 1. - Otoliths of a developed pre-fed larva of *Coregonus lavaretus* (25 mm total length). A: untreated otolith; B: after treatment using the trypsin method described in the text. Bar = 100 µm. Otoliths were mounted directly on glass slides with an instant glue and observed *in toto* with a light microscope at 320 magnification.

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